# Document made available under the Patent Cooperation Treaty (PCT)

International application number: PCT/GB05/000328

International filing date: 27 January 2005 (27.01.2005)

Document type: Certified copy of priority document

Document details: Country/Office: GB
Number: 0402123.4
Filing date: 30 January 2004 (30.01.2004)

Date of receipt at the International Bureau: 16 March 2005 (16.03.2005)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)

PCT/GB2005/000328


INVESTOR IN PEOPLE

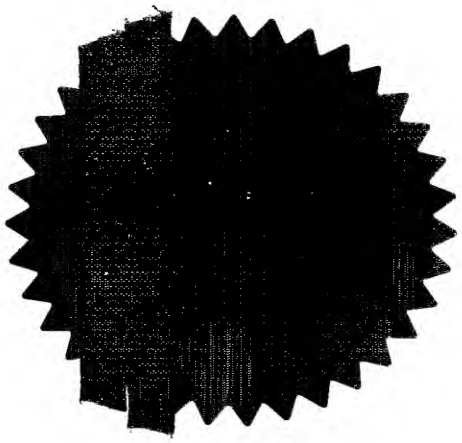
The Patent Office
Concept House
Cardiff Road
Newport
South Wales
NP10 8QQ

I, the undersigned, being an officer duly authorised in accordance with Section 74(1) and (4) of the Deregulation & Contracting Out Act 1994, to sign and issue certificates on behalf of the Comptroller-General, hereby certify that annexed hereto is a true copy of the documents as originally filed in connection with the patent application identified therein.

In accordance with the Patents (Companies Re-registration) Rules 1982, if a company named in this certificate and any accompanying documents has re-registered under the Companies Act 1980 with the same name as that with which it was registered immediately before re-registration save for the substitution as, or inclusion as, the last part of the name of the words "public limited company" or their equivalents in Welsh, references to the name of the company in this certificate and any accompanying documents shall be treated as references to the name with which it is so re-registered.

In accordance with the rules, the words "public limited company" may be replaced by p.l.c., plc, P.L.C. or PLC.

Re-registration under the Companies Act does not constitute a new legal entity but merely subjects the company to certain additional company law rules.



Signed Pandora Gerry

Dated 22 February 2005

Patents Form 1/77

[redacted]ts Act 1977
(Rule 16)

THE PATENT OFFICE
D
30 JAN 2004

The Patent Office

# 1/77

## Request for grant of a patent

*(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)*

The Patent Office
Cardiff Road
Newport
South Wales
NP10 8QQ

1. **Your reference** 444.83372/000

2. **Patent application number** *(The Patent Office will fill in this part)* 0402123.4

02FEB04 E869756-2 000027
P01/7700 0.00-0402123.4 ACCOUNT CHA

3. **Full name, address and postcode of the or of each applicant** *(underline all surnames)*

Protherics PLC
The Health and Technical Park
Runcorn
WA7 4QF

30 JAN 2004

**Patents ADP number** *(if you know it)* 8405193002

**If the applicant is a corporate body, give country/state of its incorporation** England

4. **Title of the invention** Method

5. **Name of your agent** *(if you have one)* Frank B. Dehn & Co.

**"Address for service" in the United Kingdom to which all correspondence should be sent** *(including the postcode)*

179 Queen Victoria Street
London
EC4V 4EL

**Patents ADP number** *(if you know it)* 166001

6. **Priority: Complete this section if you are declaring priority from one or more earlier patent applications, filed in the last 12 months**

| Country | Priority application number *(if you know it)* | Date of filing *(day / month / year)* |
|---|---|---|
| | | |

7. **Divisionals, etc: Complete this section only if this application is a divisional application or resulted from an entitlement dispute** *(see note f)*

| Number of earlier UK application | Date of filing *(day / month / year)* |
|---|---|
| | |

8. **Is a Patents Form 7/77 (Statement of inventorship and of right to grant of a patent) required in support of this request?**

*Answer YES if:*

*a) any applicant named in part 3 is not an inventor, or*
*b) there is an inventor who is not named as an applicant, or*
*c) any named applicant is a corporate body.*

*Otherwise answer NO (See note d)*

Yes

# Patents Form 1/77

**9. Accompanying documents: A patent application must include a description of the invention. Not counting duplicates, please enter the number of pages of each item accompanying this form:**

| | |
|---|---|
| **Continuation sheets of this form** | 0 |
| **Description** | 10 |
| **Claim(s)** | 2 |
| **Abstract** | - |
| **Drawing(s)** | 1 +1 |

**10. If you are also filing any of the following, state how many against each item.**

| | |
|---|---|
| **Priority documents** | - |
| **Translations of priority documents** | - |
| **Statement of inventorship and right to grant of a patent** *(Patents Form 7/77)* | - |
| **Request for a preliminary examination and search** *(Patents Form 9/77)* | - |
| **Request for a substantive examination** *(Patents Form 10/77)* | - |
| **Any other documents** *(please specify)* | - |

**11.** **I/We request the grant of a patent on the basis of this application.**

**Signature** Frank B Dehn & Co **Date** 30 January 2004

**12. Name, daytime telephone number and e-mail address, if any, of person to contact in the United Kingdom**

Julian Cockbain
020 7206 0600

**Warning**

*After an application for a patent has been filed, the Comptroller of the Patent Office will consider whether publication or communication of the invention should be prohibited or restricted under Section 22 of the Patents Act 1977. You will be informed if it is necessary to prohibit or restrict your invention in this way. Furthermore, if you live in the United Kingdom, Section 23 of the Patents Act 1977 stops you from applying for a patent abroad without first getting written permission from the Patent Office unless an application has been filed at least 6 weeks beforehand in the United Kingdom for a patent for the same invention and either no direction prohibiting publication or communication has been given, or any such direction has been revoked.*

**Notes**

a) *If you need help to fill in this form or you have any questions, please contact the Patent Office on 0645 500505.*
b) *Write your answers in capital letters using black ink or you may type them.*
c) *If there is not enough space for all the relevant details on any part of this form, please continue on a separate sheet of paper and write "see continuation sheet" in the relevant part(s) of the form. Any continuation sheet should be attached to this form.*
d) *If you have answered 'Yes', Patents Form 7/77 will need to be filed.*
e) *Once you have filled in the form you must remember to sign and date it.*
f) *Part 7 should only be completed when a divisional application is being made under section 15(4), or when an application is being made under section 8(3), 12(6) or 37(4) following an entitlement dispute. By completing part 7, you are requesting that this application takes the same filing date as an earlier UK application. If you want the new application to have the same priority date(s) as the earlier UK application, you should also complete part 6 with the priority details.*

83372.623

## Method

This invention relates to improvements in and relating to assay methods for testing for transmissible spongiform encephalopathy (TSE) in mammalian subjects.

Spongiform encephalopathies are a group of degenerative neurological diseases. Examples have been found in a number of mammalian species including sheep (where it is known as scrapie), cows (BSE) and humans (Creutzfeldt-Jakob disease (CJD), new variant CJD (nv CJD) and kuru). It has been reported that TSE from one species can be transmitted under laboratory conditions to mammals of another species. This crossing of species barriers by the infective agent has led to widespread concern that transfer to humans can occur as a result of ingestion of material from an infected food animal, in particular materials of bovine origin.

TSEs are characterised by a slow incubation time after which the clinical symptoms of progressive degeneration of mental state, including aggressiveness and lack of coordination, appear. Post mortems reveal a characteristic pattern of vacuolation in brain tissue due to the destruction of neural cells, and the deposition of unusual protein fibres.

Although the form of the disease found in sheep has been know for many years, spongiform encephalopathies have come to greater prominence following the appearance of BSE in cattle and of nvCJD in humans.

It is believed that the causative agent of TSE is a so-called "prion", that is an infective agent comprising protein only and no nucleic acid. In TSE, one particular protein (termed prion protein, PrP) has been identified as the infective agent. PrP is a naturally occurring cellular protein that exists in two isoforms which differ in their tertiary structure and as a result

may be distinguished by their response to enzymatic degradation, e.g. by proteinase K. Thus the non-infectious isoform niPrP is wholly digested by proteinase K while the infectious isoform iPrP is degraded to leave a detectable polypeptide residue PrP27-30.

The amino acid sequences for many mammalian PrP are known and accessible, for example on SwissProt. The amino acid sequences for the residue PrP27-30 are likewise known. There is a high degree of homology between the different mammalian PrP sequences.

Several companies have developed post mortem diagnostic tests for TSE, generally based on the use of antibodies which bind to PrP27-30 deriving from a proteolytically digested brain tissue sample.

One such assay available from Enfer, Dublin, Ireland, uses the technology described in EP-B-616613. More particularly the Enfer assay uses two polyclonal antibodies raised against immunogenic conjugates of polypeptide sequences corresponding (i) to a section of PrP27-30 and (ii) to a section of PrP outside the PrP27-30 section. These sections are referred to in EP-B-616613 as Vc and Va respectively.

While the commercial assays have met with some success there is a continuing need for improved TSE assays and in particular for assays that may be performed ante-mortem or which do not require brain tissue samples.

The present invention provides just such an improved assay.

Thus viewed from one aspect the invention provides an assay method for detecting infectious prion protein in a sample from a mammalian subject, said method comprising: obtaining a prion protein containing sample from said subject; contacting said sample with an agent which serves to digest non-infectious prion protein and to partially digest infected prion protein to yield a

prion protein polypeptide residue; contacting the digested sample with an antibody capable of binding to a polypeptide having the amino acid sequence Vc

(Gly-Gly-Gly-Trp)-Gly-Gln-Gly-Gly-$R_1$-$R_2$-His-$R_3$-Gln-Trp-Asn-Lys-Pro-$R_4$-Lys-Pro-Lys-Thr-$R_5$-$R_6$-Lys(-His-$R_7$-Ala-Gly) (Vc)

(wherein $R_1$ is either Gly or absent;
$R_2$ is either Thr or Ser;
$R_3$ is an amino acid residue selected from Gly, Ser and Asn;
$R_4$ and $R_5$ are each independently either Asn or Ser;
$R_6$ is an amino acid residue selected from Met, Leu and Phe;
$R_7$ is either Val or Met; and wherein one or more residues within brackets may be present or absent with the proviso that if they are present they are attached to the rest of the peptide in sequence); and detecting conjugates of said antibody and said prion protein polypeptide residue; characterized in that the detection of said conjugates comprises chemical, biological or biochemical amplification, especially preferably biochemical or biological amplification, of a detectable species and detection of the amplified species.

The antibody capable of binding to Vc is preferably an antibody raised against an immunogenic conjugate of a synthetic polypeptide of amino acid sequence Vc (or more preferably Vc' as described below), e.g. by vaccination of a mammal therewith and collection of sera or Vc-binding IgG.

The prion protein containing sample used in the method of the invention may be a sample of any body tissue, fluid or material which contains prion proteins, e.g. muscle, tonsil, brain, blood, urine, faeces, etc. The sample may also be blood, serum or plasma from blood banks, blood products (e.g. coagulation factors), tissue

products, culture media containing mammalian products (e.g. BSA), pharmaceutical components derived from mammalian species (e.g. heparin), etc. For post-mortem testing, brain tissue will desirably be used due to its high prion content. For ante-mortem testing the sample is preferably a biopsy tissue sample, blood, urine or faeces. The sample is preferably pretreated to lyse any cells therein, e.g. by homogenization or other tissue disruptive methods.

The sample is then contacted with the prion protein digesting agent, e.g. proteinase K, under conditions and for a period sufficient for digestion of the non-infectious prion protein. Such treatments and treatment conditions and durations are well known in the art.

If desired the sample may be treated before and/or after digestion to separate out sample components and/or digestion products other than undigested or partially digested prion protein, e.g. by centrifugation, chromatography, etc.

Following digestion, the sample is contacted with the Vc-binding antibody. This may be prepared as described in EP-B-616613. Particularly preferably the antibody is prepared using a conjugate of a polypeptide of sequence

GQGGSHSQWNKPSKPKTNMKHVGC (Vc')

with an immunogenic carrier, e.g. tetanus toxoid, ovalbumin, etc. Conjugation may be effected using a standard linking agent, e.g. m-maleimido-benzoyl-N-hydroxy sulphosuccinimide easter (SMBS), etc. The antibody is preferably polyclonal. Standard antibody production techniques may be used.

The Vc-binding antibody may be immobilized on a substrate (e.g. a flat surface, optionally superparamagnetic beads, rods, meshes, tubes, etc) using conventional protein immobilization techniques.

Alternatively, a non-immobilized Vc-binding antibody may be used.

The precise series of steps in the detection stage of the assay method will depend on whether an immobilized or non-immobilized Vc-binding antibody is used and on the technique selected for chemical, biological or biochemical amplification.

By chemical amplification it is meant that a non-biochemical chemical reaction (e.g. a reaction catalysed by a chemical substance not normally found in a biological environment) is used to generate a detectable species, the presence or absence of which is indicative of the presence of antibody:prion protein polypeptide residue conjugates.

By biological amplification it is meant that a microorganism is used to generate a detectable species (e.g. chemical substance, microorganisms, etc), the presence or absence of which is indicative of the presence of antibody:prion protein polypeptide residue conjugates.

By biochemical amplification it is meant that a biochemical reaction (e.g. an enzymatic reaction or a nucleic acid amplification such as PCR) is used to generate the detectable species (e.g. chemical substance), the presence or absence of which is indicative of the presence of antibody:prion protein polypeptide residue conjugates.

The material which is amplified or which causes the amplification to occur may be conjugated to the Vc-binding antibody or to a further agent capable of binding to the antibody:residue conjugates, e.g. a second antibody. Where it is conjugated to the Vc-binding antibody, its ability to function may be unaffected by conjugation to the PrP27-30 or it may be activated or deactivated by such conjugation. As a result, unreacted antibody may or may not have to be separated from antibody:residue conjugates. This is

conventional in immunoassay procedures and the person of ordinary skill will readily appreciate the steps that should be taken. Where the material which is to be amplified or which causes amplification is separate from the Vc-binding antibody, it will generally be necessary to separate unconjugated antibody from the antibody:residue conjugates. Again this is conventional in immunoassay procedures and the person of ordinary skill will readily appreciate the steps that should be taken.

The material which is amplified or which causes amplification may comprise more than one component. In this case, one of the components may be conjugated to the Vc-binding antibody and the other to a separate agent capable of binding to the antibody:residue conjugate. Once again it will be clear to the person of ordinary skill whether separation of unconjugated Vc-binding antibody from antibody:residue conjugates is required. Where such a two or more component system is used, it is preferably the case that the different components together create a different amplification effect from that achievable with the single components on their own, e.g. they may be catalysts (e.g. enzymes) that catalyse different stages of a multistage reaction or they may be viral agents having different effects on the same or different target microorganisms.

Where a second binding agent is used in the assay method of the invention this is preferably also an antibody. However other binding agents may be used if desired.

The term antibody as used herein, unless the content dictates otherwise, may be an antibody as such or a functional fragment (e.g. a Fab fragment) thereof, a single chain antibody or an oligomeric antibody construct. Such materials may be produced in conventional fashion.

To avoid unnecessary false negatives, the assay

method of the invention also preferably involves testing a portion of the original sample for PrP content. This may be done conventionally, e.g. using PrP binding antibodies as for example in the commercial BSE tests. In this case, the PrP binding antibody should be capable of binding to iPrP and/or niPrP or fragments thereof exposed by denaturation or partial digestion. Such antibodies are described in the literature.

However in a preferred embodiment, a Va-binding antibody is used on non-PK-digested samples, i.e. analogously to the Vc-binding antibodies with the omission of the niPrP digestion step. Particularly preferably the antibody used is a Va-binding antibody as defined below. Especially preferably such an antibody is one raised against an immunogenic conjugate of a synthetic polypeptide of amino acid sequence Va (or more preferably Va' as described below), e.g. by vaccination of a mammal therewith and collection of sera or Va-binding IgG.

By a Va-binding antibody is meant one capable of binding to a polypeptide of sequence

(Pro-Gly-Gly-$R_8$)-Trp-Asn-Thr-Gly-Gly-Ser-Arg-Tyr-Pro-Gly-Gln-Gly-Ser-Pro-Gly-Gly-Asn-Arg-Tyr-Pro-Pro-Gln-Gly-(Gly-$R_9$-$R_{10}$-Trp) (Va)

wherein $R_8$ and $R_9$ are each independently either Gly or absent;
$R_{10}$ is either Gly or Thr; and wherein one or more residues within brackets may be present or absent with the proviso that if they are present they are attached to the rest of the peptide in sequence.

This may be prepared as described in EP-B-616613. Particularly preferably the antibody is prepared using a conjugate of a polypeptide of sequence This may be prepared as described in EP-B-616613. Particularly preferably the antibody is prepared using a conjugate of

a polypeptide of sequence

GGWNTGGSRYPGQGSPGGNRYPPQGGGC (Va')

with an immunogenic carrier, e.g. tetanus toxoid, ovalbumin, etc. Conjugation may be effected using a standard linking agent, e.g. SMBS. The antibody is preferably polyclonal. Standard antibody production techniques may be used.

Where an ante-mortem TSE assay result is positive, it is preferable to treat the subject with a therapeutic agent comprising a Vc-binding antibody. This may be the antibody alone or may be a conjugate of the antibody with an agent which serves to hinder the transformation of niPrP to iPrP or to degrade the iPrP. In the case of human TSE, the therapeutic antibody is preferably a selected antibody (i.e. an anti-Vc or anti-Va antibody as defined above). Administration is preferably by injection or infusion, e.g. into the CSF. Dosages may be determined conventionally using TSE infected animal models. Such therapeutic use forms a further aspect of the present invention.

Viewed from a further aspect the invention provides a kit for use in the assay method of the invention, said kit comprising:

(i) a Vc-binding antibody;

(ii) optionally and preferably a Va-binding antibody;

(iii) optionally and preferably proteinase K;

(iv) a material capable of chemical, biological or biochemical amplification, preferably biological or biochemical amplification, and detection or of causing chemical, biological or biochemical amplification, preferably biological or biochemical amplification, of a detectable species, said material optionally being conjugated to antibody (i); and

(v) optionally and preferably instructions for the performance of said assay method

While the material (iv) may be an enzyme or other catalyst it is preferably a substance which is itself replicated in the amplification procedure.

The invention will now be described further with reference to the following non-binding Examples:

Example 1
Sample Preparation

Initial tissue disruption using appropriate methods such as homogenisation in a detergent buffer using pestle and centrifuge tube to produce a crude homogenate which may be clarified by centrifugation. The tissue homogenate sample is diluted to a working concentration in a suitable buffer in readiness for Proteinase K digestion.

Example 2
Prion Protein Digestion

To distinguish iPrP from niPrP the detection systems rely on the relative resistance of iPrP to digestion by the enzyme proteinase K (PK) (see Kretzschmar, *Clin Lab Med*. P109-128 (2003)). Typical conditions of PK treatment would be, diluted homogenate sample split into two equal portions and PK added to one portion and incubated accordingly (e.g. for 30 mins at 37°C).

Example 3
Vc-Binding Antibody Addition

Serum or purified IgG are prepared from the blood of animals immunised with Vc peptide conjugate vaccine. These sera would then be diluted to a working concentration, contacted with the PK digested

homogenised sample and incubated accordingly (e.g. for 1 hour at 20-25°C).

Example 4

Signal Amplification and Detection

Signal amplification would typically require applying biochemical techniques, molecular or conjugated secondary antibody techniques. One example would be the incubation of an antibody conjugated with biotin molecules (e.g. for 1 hour at 20-25°C) amplified by the addition of Avidin (biotin binding agent) conjugated to horse radish peroxidase (e.g. for 1 hour at 20-25°C) and detection using a chromogenic substrate, read at a specific absorbance on a spectrophotometer.

Example 5

Antibody Efficacy

Vc-binding and Va-binding polyclonal antibodies prepared by animal immunization with antigenic conjugates of Vc' and Va' respectively were contacted with brain homogenate prepared as described above from CJD infected and non-infected human brain with and without proteinase K digestion. The samples were then subjected to Western blot analysis and the results are shown in accompanying Figures 1 and 2. Figures 1 and 2 are for Va-binding and Vc-binding antibodies respectively. In each Figure, lane 1 is uninfected homogenate, lane 2 is PK treated uninfected homogenate, lane 3 is infected homogenate, and lane 4 is PK-treated infected homogenate.

Claims:

1. An assay method for detecting infectious prion protein in a sample from a mammalian subject, said method comprising: obtaining a prion protein containing sample from said subject; contacting said sample with an agent which serves to digest non-infectious prion protein and to partially digest infected prion protein to yield a prion protein polypeptide residue; contacting the digested sample with an antibody capable of binding to a polypeptide having the amino acid sequence Vc

(Gly-Gly-Gly-Trp)-Gly-Gln-Gly-Gly-$R_1$-$R_2$-His-$R_3$-Gln-Trp-Asn-Lys-Pro-$R_4$-Lys-Pro-Lys-Thr-$R_5$-$R_6$-Lys(-His-$R_7$-Ala-Gly) (Vc)

(wherein $R_1$ is either Gly or absent;
$R_2$ is either Thr or Ser;
$R_3$ is an amino acid residue selected from Gly, Ser and Asn;
$R_4$ and $R_5$ are each independently either Asn or Ser;
$R_6$ is an amino acid residue selected from Met, Leu and Phe;
$R_7$ is either Val or Met; and wherein one or more residues within brackets may be present or absent with the proviso that if they are present they are attached to the rest of the peptide in sequence); and detecting conjugates of said antibody and said prion protein polypeptide residue; characterized in that the detection of said conjugates comprises chemical, biological or biochemical amplification of a detectable species and detection of the amplified species.

2. A method as claimed in claim 1 wherein said subject is human, preferably animate.

3. A method as claimed in either of claims 1 and 2 for

detecting infectious prion protein associated with CJD, nvCJD or kuru.

4. A kit for use in the assay method of any one of claims 1 to 3, said kit comprising:

(i) a Vc-binding antibody;

(ii) optionally a Va-binding antibody;

(iii) optionally proteinase K;

(iv) a material capable of chemical, biological or biochemical amplification and detection or of causing chemical, biological or biochemical amplification of a detectable species, said material optionally being conjugated to antibody (i); and

(v) optionally instructions for the performance of said assay method.

5. The use of a iPrP binding antibody in the manufacture of a medicament for use in the treatment of human TSE.

Fig. 1

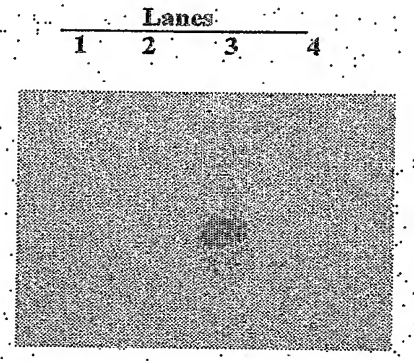



Fig. 2